Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claims 1-8 (Cancelled).

9(Currently amended). A method for detection of differential expression of natural antisense messenger RNA (mRNA), comprising:

(a) separately obtaining polyA-mRNA-A molecules from cell population A and polyA-mRNA-B molecules from cell population B;

(b) separately generating by a reverse transcription enzyme a population of single-stranded cDNA-A molecules from polyA-mRNA-A and a population of single-stranded cDNA-B molecules from polyA-mRNA-B, wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-B molecules comprises a specific bacteriophage RNA polymerase promoter region close to its 5' terminus;

(c) incubating the combined populations of single-stranded cDNA-A molecules and single-stranded cDNA-B molecules, under conditions allowing hybridization of sense cDNA molecules with antisense cDNA molecules, wherein each single-stranded antisense cDNA molecule that hybridizes has a segment

complementary to the sense cDNA molecule and hybridizes thereto to form a hybrid molecule with a double-stranded segment;

(d) treating the hybrid molecules with a DNA polymerase having a 5' to 3' polymerase activity and a 3' to 5' exonuclease activity to remove single-stranded non-hybridized segments of the hybrid molecule from 3' to 5' and to extend the double-stranded segment of the hybrid molecule 5' to 3' over an adjacent single-stranded segment as template, thereby forming a double-stranded molecule having the RNA polymerase promoter region close to one terminus;

(e) using the double-stranded molecule as a template for the specific RNA polymerase to produce a population of RNA molecules;

(f) labeling with a first label the RNA molecules produced in step (e);

(g) labeling with a second label as control the polyA-mRNA-A molecules and/or the polyA-mRNA-B molecules of step (a);

(h) mixing labeled RNA molecules from steps (f) and (g) and hybridizing them to a DNA microarray; and

(i) identifying the genes on the microarray which are preferentially labeled with the labeled RNA molecules of step (f), wherein the genes so identitifed on the microarray are detected as differentially ~~expressing~~ expressed natural antisense mRNA.

10(Previously presented). The method according to claim 9, wherein the specifc bacteriophage polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase, and SP6 RNA polymerase.

11(Previously presented). The method according to claim 9, wherein following step (b) the cDNA-B is modified in order to resist the 3' to 5' exonuclease activity of step (d).

12(Previously presented). The method according to claim 11, wherein in step (d) the 3' terminus of the cDNA-B is modified.

13(Previously presented). The method according to claim 11, wherein in step (d) the entire cDNA-B is modified.

14(Previously presented). The method according to claim 12, wherein 3' terminus of the cDNA-B is modified by addition of a nucleotide analog.

15(Previously presented). The method according to claim 13, wherein the entire cDNA-B is modified by incorporation of nucleotide analogs.

16(Previously presented). The method according to claim 9, wherein the first label of step (f) is Cy3, and the second label of step (g) is Cy5.

17(Previously presented). The method according to claim 9, wherein the first label of step (f) is Cy5, and the second label of step (g) is Cy3.

18(Previously presented). A method for detection of differential expression of natural antisense messenger RNA (mRNA), comprising:

(a) separately obtaining polyA-mRNA-A molecules from cell population A and polyA-mRNA-B molecules from cell population B;

(b) separately generating by a reverse transcription enzyme a population of single-stranded cDNA-A molecules from polyA-mRNA-A and a population of single-stranded cDNA-B molecules from polyA-mRNA-B, wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-A molecules comprises close to its 5' terminus a sequence identical to an amplification primer used in step (e) and wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-B molecules comprises a specific bacteriophage RNA polymerase promoter region close to its 5' terminus;

(c) incubating the combined populations of single-stranded cDNA-A molecules and single-stranded cDNA-B molecules, under conditions allowing hybridization of sense cDNA molecules with antisense cDNA molecules, wherein each single-stranded antisense cDNA molecule that hybridizes has a segment complementary to the sense DNA molecule and hybridizes thereto to form a hybrid molecule with a double-stranded segment;

(d) treating the hybrid molecules with a DNA polymerase having a 5' to 3' polymerase activity and a 3' to 5' exonuclease activity to remove single-stranded non-hybridized segments of the hybrid molecule from 3' to 5' and to extend the double-stranded segment of the hybrid molecule 5' to 3' over an adjacent single-stranded segment as template, thereby forming a double-stranded molecule having the RNA polymerase promoter region close to one terminus;

(e) amplifying the double-stranded molecule of step (d) using a thermostable polymerase and a first amplification primer identical to the sequence used in step (b) and a second amplification primer identical to the specific bacteriophage RNA polymerase promoter region of step (b);

(f) using the double-stranded molecules so produced as a template for the specific RNA polymerase to produce a population of RNA molecules;

(g) labeling with a first label the RNA molecules produced in step (f);

(h) labeling with a second label as control the polyA-mRNA-A molecules and/or the polyA-mRNA-B molecules of step (a);

(i) mixing labeled RNA molecules from steps (g) and (h) and hybridizing them to a DNA microarray; and

[[(i)]] (j) identifying the genes on the microarray which are preferentially labeled with the labeled RNA molecules of step (g).

19(Previously presented). The method according to claim 18, wherein the specifc bacteriophage polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase, and SP6 RNA polymerase.

20(Previously presented). The method according to claim 18, wherein following step (b) the cDNA-B is modified in order to resist the 3' to 5' exonuclease activity of step (d).

21(Previously presented). The method according to claim 20, wherein in step (d) the 3' terminus of the cDNA-B is modified.

22(Previously presented). The method according to claim 20, wherein in step (d) the entire cDNA-B is modified.

23(Previously presented). The method according to claim 21, wherein 3' terminus of the cDNA-B is modified by addition of a nucleotide analog.

24(Previously presented). The method according to claim 22, wherein the entire cDNA-B is modified by incorporation of nucleotide analogs.

25(Previously presented). The method according to claim 18, wherein the first label of step (g) is Cy3, and the second label of step (h) is Cy5.

26(Previously presented). The method according to claim 9, wherein the first label of step (g) is Cy5, and the second label of step (h) is Cy3.